

IV. PATHOLOGY

Cytokines profile and micronutrients in plasmodium vivax infection

Malaria is one of the leading causes of morbidity and mortality worldwide, with an estimated 350 to 500 million new cases each year. Despite wide reputation as the benign parasite, P.vivax is nevertheless associated with severe complication and death. Relapses due to P.vivax can occur even after 3 to 5 Y and may cause death as a result of high parasitemia (2%) after anemia or ruptured spleen and thrombocytopenia or rarely cerebral malaria. There is no information about cytokine profile in P.vivax though it is more commonly prevalent infection in India, hence the present study was carried out with the following objectives.

OBJECTIVES

To assess circulating IL-2, IFN γ , IL12, IL10, IL-4 and TNF α concentrations in patients with mild and severe P. Vivax infection and correlate with serum, zinc and vitamin A.

SUBJECTS & METHODS

Patients attending the out patients clinic, Hospital for tropical diseases, Hyderabad, during the month of August and September, who were diagnosed to be suffering from malaria on the basis of peripheral blood smear examination, aged between 20 to 40Y were enrolled for the study. Patients recruited for the study were classified into 2 groups based on the density of parasites in blood. Patients with 1-2 parasites in occasional high power field (HPF) and those with more than 2-10 parasites in all HPF were classified as mild/moderate and those with high density of parasitemia with poor clinical conditions (vomiting, dehydration and temperature, $>39^{\circ}\text{C}$) were classified as severe. Finally, there were 15 mild/moderate and 16 severe cases of malaria. Ten apparently normal subjects were selected randomly for the control group. Patients who received recent anti malarial treatment and those with other systemic infections were excluded from the study. After collecting a venous

blood sample, the patients received treatment as outpatients with sulfadoxin and pyrimethamin. The Institute's Scientific Advisory Committee approved the study.

Work done during the year 2004-05

Five ml of venous blood samples from each were collected into sterile EDTA-containing vacutainer, tubes. Plasma was separated immediately and frozen in -70°C to avoid denaturation of cytokines, which were quantified by sandwich ELISA. Sensitivity of detection for the cytokine assay was as follows: IL-2, 5 pg/ml; IFN γ , 5 pg/ml; and IL-4, 0.5 pg/ml. As IL12 and IL10 are known to be regulatory cytokines and TNF α is suggested to be associated with anemia in malaria, these three cytokines were determined again in the same patients after recovery from malaria.

Serum zinc was determined by atomic absorption spectrophotometry and vitamin A was assayed by HPLC. Log transformed data were compared between groups using students t test. When data remained skewed after log transformation the Mann Whitney U test was used.

The mean age of patients was similar in the three groups. The mean number of days the patients had pyrexia before collection of blood was similar in mild/moderate ($4.2 \pm 0.5\text{d}$), and severe ($4.4 \pm 0.4\text{d}$) malaria. The results were as follows.

1. The initial hemoglobin concentration was significantly lower in mild and severe malaria (12.23 g/dl) compared to normal population and decreased to 10.63 g/dl within 1 week. Packed cell volume (PCV) percent was significantly lower in patients compared to controls and decreased significantly from the initial level in both mild and severe malaria.
2. Serum zinc ranged from 40 to 100 $\mu\text{g}/\text{dl}$ with a mean $\pm\text{SE}$ of $63.8 \pm 2.6\mu\text{g}/\text{dl}$, which was much less than the reported normal range (70-120 $\mu\text{g}/\text{dl}$). Of the 36 cases of malaria 61% had

zinc deficiency and the rest (14) had equal to or more than 70µg/dl. The mean zinc levels were comparable between the groups in malaria though mild malaria group had low zinc concentrations (Table 19).

Table 19. P.vivax and Micronutrients

<i>P.vivax</i>	Hb gms/dL	Zn µg/dl	Vitamin A µg/dl
Mild	12.3± 0.88	59.09± 2.73	25.10± 3.52
Severe	12.26± 0.5	68.83± 8.88	21.24± 5.01

Values are Mean ± SE

3. Serum Vitamin A concentration ranged from 8 to 55 µg/dl and was less than the observed range (20 to 70µg/dl) in normal adults. The total mean ± SE of vitamin A was 29.3±2.41 µg/dl. Of the 36 patients of malaria, 25 had normal vitamin A (>20µg/dl), while 11 had less than 20µg/dl. Vitamin A levels did not correlate with severity of malaria.
4. The mean concentration of IL2 was higher in the mild (16.9±5.19 pg/ml) malaria compared to severe (11.6±3.33 pg/ml), while IFN γ was significantly higher in the severe malaria (619.7±230.97). IL4 was 2.4±1.0 and 3.8±1.99 pg/ml in the mild and severe malaria respectively (Table 20).
5. The IL12 ranged from 85.2 to 464 pg/ml and the mean was 289.5±67.94 pg/ml in mild

malaria and was comparable to severe malaria (240.2±46.24). After one week with the clearance of parasites the IL12 decreased by fifty percent in the mild malaria, while same level was maintained in the severe malaria thus showing the association of serum IL12 with parasite clearance.

Table 20. Cytokine profile in P.vivax infection

Cytokines	Mild	Severe
IL-2 pg/ml	16.9 ± 5.19(14)	11.6±3.33(6)
IFNγ pg/ml	177.3 ± 40.98(14)	619.7±230.7(6)
IL4 pg/ml	2.8±1.0(14)	3.8±1.99(6)
IL-12 pg/ml	289.5±67.94(5)	240.2±46.24(10)
IL-10 pg/ml	452.76±334.33(5)	279.1±121.8(10)
TNFα pg/ml	32.64±3.65(5)	44.3±19.3(10)

Values are mean SE Numbers in parenthesis

6. The initial plasma level of IL10 was 452.7±334.33 in the mild malaria that was significantly higher than the severe malaria (279.1±121.8). However, after 1 week there was a 50% increase in IL10 in the severe malaria while it remained the same in mild malaria. The plasma level of TNF α was 32.6 ±3.65pg/ml, which was maintained even after 1week in the mild malaria. However, TNF α increased 3 fold from the initial value of 44.3±19.36 pg/ml in the severe malaria.